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(71) Applicant *(for all designated States except US)*: PICRON MICROARRAY AB [SE/SE]; Uppsala Science Park, S-751 83 Uppsala (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: MALMQUIST, Mats [SE/SE]; Hurtigs gata 63, S-754 39 Uppsala (SE).

(74) Agent: HOLMBERG, Martin; Bergenstråhle & Lindvall AB, P.O. Box 17704, S-118 93 Stockholm (SE).

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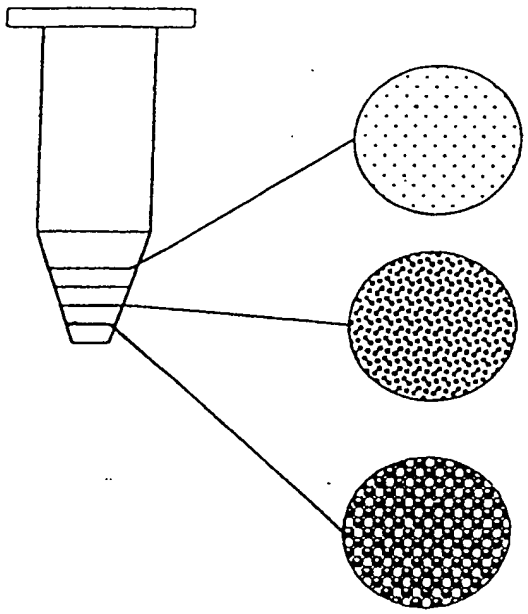
(57) Abstract

A particulate or dynamic microarray system is produced by immobilising reactant molecules on particles, arranged in at least three distinct groups corresponding to the number of reactants used. Preferably, the groups are separated by intervening groups without immobilised reactant molecules or with specific signal molecules. Further, the particles are suspended in a medium which can be solidified subsequent to the reaction or reactions between analytes and immobilised reagents. The inventive system offers better kinetic properties than presently used spatially static microarrays, where reactant molecules are immobilised e.g. on gels or chips.



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Microarray system and process for performing biochemical reactions

The present invention concerns a new concept for performing biochemical reactions, and a system and products for this purpose. The present invention further discloses a process for the manufacture of said products. In particular, the present invention concerns a dynamic microarray system, comprising a particulate carrier matrix, divided in distinct groups.

Background of the invention

When a sample is available in larger amounts, it can be divided into several, identical sample aliquots. These aliquots can then be subjected to a multitude of tests, for example by dispensing the aliquots in microtiter plates, where each well has the function of one reaction vessel and represents one reaction. Other samples, available in limited amounts, have to be subjected to multiple analysis simultaneously, performed in one and the same reaction vessel. This will be referred to as "multiplex analysis" in the following description.

The rapidly developing technology, known as microarray technology, has recently become a promising tool for laboratories involved in biochemical and gene technological research and development. Microarrays are used for multiplex analysis of a single sample. Typical applications are high throughput screening for pharmacological compounds, medical diagnostics based on gene technological methods, gene discovery, gene expression monitoring, gene mapping and sequencing of deoxyribonucleic acid (DNA) molecules. Conventional and presently used formats for multiplex analysis, of single samples, are dot blot analysis and various types of techniques referred to as dipstick-analysis, fragment analysis etc.

Currently, new methods for producing such microarrays are developed. In the field of gene technology, microarrays are produced by so-called *in situ* (on-chip) synthesis of oligomers of DNA or peptide nucleic acid (PEA). Another approach is to use the piezoelectric printing method utilised in inkjet printers to spot and immobilise oligonucleotide DNA or PNA molecules to a solid glass or nylon substrate. Sample nucleic acid is directly or after amplification e.g. by the polymerase chain reaction (PCR) hybridised to complementary sequences on the array. The hybridisation is normally detected by laser scanning using fluorescent labels incorporated into the hybridising molecules. A review of the state-of-the-art

of DNA related microarray technology is given by Ramsay in Nature Biotechnology, 16,1998, 40-44.

The principle of applying microarray technology in applications as given above is to specify a set of individual locations with respect to their unique co-ordinates in a two dimensional matrix. At each location, some event of specific interest may occur and be recorded. Since the unique co-ordinate of each location is known, these events can be observed individually and compared with the nature of events at other locations. An event in this context is typically a chemical reaction of specific interest, for instance a process of chemical binding of molecules to each other or else the formation of various chemical complexes. This may for instance be hybridisation, polymerisation or degeneration processes occurring either spontaneously or induced by other processes. Any of the processes involved may be of photochemical or electrochemical nature.

Regardless of chemistry, current microarray technology use the discrete locations at a specific solid surface area to give the individual identification required to separate different observations from each other. These discrete locations are referred to as "dots" and the solid surface area on which these dots are located are denoted the "matrix". One single two-dimensional x-y-co-ordinate system covers the matrix and thus provides the reference required for giving each dot its unique value of x and y. One "array" is according to conventional microarray technology defined as the complete set of dots in such two-dimensional matrix.

There are specific advantages with keeping the distances between each dot in a matrix as short as possible. One advantage is that chemical concentration gradients over the array are equilibrated more quickly if the dot-to-dot distances are as short as possible. Another is that the overall size of instruments based on microarray technology may be reduced if the so-called density of dots, that is the number of dots per area unit, is high. In presently used systems a density of around 20 - 30.000 probes per cm^2 has been reached and a theoretical limit of 10^6 is discussed. There is, however, a lower length limit for distances between dots which is defined by the degree of resolution of the means used to obtain discrete observation over the matrix.

As a consequence of both the efforts of keeping distances between dots short and to obtain a high degree of resolution, it is important that the solid surface over which the matrix is laid is rigid. This because obscure positions of the dots in relation to the reference matrix may lead to confused identity of the specific observation events. Thus microarrays

conventionally used are produced on such rigid solid surfaces. Thus, these microarrays can be described as spatially static microarrays, and are in the present text referred to as "static" microarrays.

However, there is a problem with static microarrays. This is due to slow chemical reactions. The kinetic properties of chemical reactions occurring in a liquid phase corresponds to the time it takes for the reactant molecules to become positioned appropriately with respect to each other so that the molecular interactions between the reactants, required for the specific chemical reaction, occur. It is a fact that the more quickly the reactant molecules involved move in the chemical environment, on average the quicker chemical reaction. This is referred to as quick kinetics in contrast to slow kinetics. If one of the reactant molecules is immobilised in a specific position, the kinetics is slower compared to if all reactant molecules are mobile.

Unfortunately, slow kinetics is a significant drawback in most applications of microarrays. Slow kinetics is related to low resolution of individual observations as well as low sensitivity of assays based on chemical binding events. If used for sequencing by hybridisation, low resolution may lead to obscure or erroneous sequence determination. If used for differential mRNA-display analysis, slow kinetics may lead to wrong conclusions being drawn or a false clinical diagnosis being made. If used for high-throughput screening of pharmaceutical leads, important drug target molecules may be neglected due to slow kinetic properties.

In microarray based approaches it is relevant to be able to separate a manifold, e.g. hundred or thousands of different chemical reactions from each other. Unfortunately, different chemical reactions are not easy to observe and discern from other chemical reactions occurring simultaneously in the same solution. A first chemical reaction may be for instance hybridisation between a set of identical nucleic acid molecules and other nucleic acid molecules being complementary to this set. A second chemical reaction may be hybridisation between a second set of nucleic acid molecules and their complementary counterparts.

Prior art

U.S. 4,721,681 by Lentrichia *et al.* discloses a binary system for a so called competitive or inhibition assay, comprising first and second particles with complementary binding pair members. In an embodiment of the disclosed invention, the first, heavy particles have adsorbed a suitable antigen and the second, light particles have adsorbed the

corresponding antibody. After some or complete immunochemical reaction, centrifugation will cause the heavy particles and any light/heavy combinations to migrate outward in the centrifugal field. By waiting until such particles have migrated outwardly beyond a light path locus, only a concentration of light particles should remain suspended in the reaction medium. By measuring absorbance or light scattering, a measure related to the concentration of light particles can be obtained. This value is then related to the analyte concentration with the help of a standard curve previously generated.

The concept of a plurality of bead groups is disclosed in WO97/35201 by Markman and Paleyov. In the description and examples, each bead group is defined as having at least one common identifying physical characteristic. However, only bead shape, size and colour are mentioned. Further, the bead size is specified by an upper limit of 0.5 to 10 μm. Additionally, the concept disclosed in WO 97/35201 entails a sorter, adapted to sort the beads according to their respective, identifying physical characteristics. The sorter is further defined as a fluorescence-activated cell sorting apparatus, which sorts the beads according to fluorescent light emission therefrom.

In view of the above, it is apparent that there still exists a need for an identification system for individual chemical reactions, said system being easily automated and having better kinetic properties than presently known microarray methods. Also, the issue of space limitations and restricted accessibility of the reactants, currently hampering the use of two-dimensional arrays, needs to be addressed.

Summary of the invention

The present invention solves the above outlined problems and shortcomings of the presently used microarray systems by introducing a novel concept for performing microarray type assays according to the attached claims. The microarray system, method and kit according to the present inventive concept offer not only better kinetic properties, but can also simplify the handling of reactants and samples, provide faster and easier detection of the result and make it possible to integrate the determination with conventional laboratory practise, such as incubation and centrifugation of reaction mixtures.

Brief description of the drawings

The present invention will be described in closer detail in the following description, examples and drawings, in which

Figure 1 shows schematically how different positions represent distinct particle groups, which carry different reagents,

Figure 2 illustrates the principle described in production example 1,

Figure 3 illustrates the principle described in production example 2, and

Figure 4 illustrates the principle described in production example 3.

Description of the invention

Particle suspensions in solutions, for instance aqueous solutions, show similarities with true solutions in that the particles move more or less freely in the solution. Further, particle suspensions expose a large total surface area being in contact with the aqueous solution. The present invention is based on the idea, that if individual particles in a particle suspension at the same time could bind one or more reactants and if the particles could be unambiguously identified, i.e. separated by any means from other particles, it would be possible to develop a microarray-based on such a system.

The present invention comprises a microarray system comprising reactants and a carrier matrix, characterized in that said carrier matrix comprises particulate matter in a number of at least three groups, each group having a distinct property and that said reactants are immobilised to said particles with each one reactant corresponding to one specific group with its specific property. The specific property is chosen among particle size or diameter, particle density, sedimentation properties, magnetic properties, radioactivity, colour, chemical affinity or a combination thereof.

According to a preferred embodiment, the chosen property is particle density.

The present invention further comprises a system as above, characterized in that density groups with immobilised reactants are separated by intermediate density groups without immobilised reactants.

Preferably, the density groups carrying immobilised reactants are separated by intermediate density groups with specific signal molecules, for example colorimetric agents, fluorophores or luminophores.

One embodiment of the present invention is a kit for the performing of multiplex analysis, characterized in that said kit comprises a number of particle groups with distinct

densities, each particle group carrying a reagent, bound to the particles in that group. The different particle groups may be delivered separately, in separate containers and in an environment facilitating storage. This would facilitate the "tailoring" of specific tests by combining particle groups with immobilised reactants in accordance with the desired determination. When observing the simple criteria, that the resulting test must contain discrete groups with respect to the densities or chosen property. This would offer a great flexibility for the user, compared to the use of static microarrays.

It is also conceivable, that a set of particle groups with corresponding reagents is delivered together in a "ready-to-use" kit. A kit, according to the invention, may further comprise a reaction medium, one or more suitable reaction vessels, instructions for performing the analysis and instructions for interpreting the results.

According to a preferred embodiment of the invention, the reaction medium is a composition which has the following properties:

- it is in a liquid state during the performing of the analysis, not imposing restrictions on the mobility of the particulate carrier matrix, and
- after the necessary reactions have taken place and after performing the necessary separation of the distinct groups, such as separation by centrifugation, the medium can be forced to solidify and thus preserving the order of the separated groups.

Optionally, the medium is a buffered solution having a composition preserving, protecting or sustaining the functional entities subject to or used in the determination. When it is desirable to include a pre-treatment step, this can be incorporated in the analysis by adapting the medium. According to this embodiment, the medium consists for example of a lysis buffer. In a process according to this specific embodiment, a cellular sample is mixed with the medium containing labelled particles, vortexed and centrifuged. Cellular debris is separated during centrifugation and the reactants bind to the particles. The result of the analysis is interpreted during or after the centrifugation step, by determining which particle groups - containing specific reagents – have reacted with the compounds present in the sample.

One possibility, according to the present invention, is to use an aqueous solution which, after completion of the centrifugation. is frozen by lowering the temperature. Another suitable medium is a medium containing polymeric or polymerisable components. Cross

linking or polymerisation can then be initiated e.g. through irradiation, without disturbing the order of the separated groups.

Another medium used according to the present invention is a liquid which is viscous or highly viscous at room temperature and thin or mobile at an elevated temperature. Consequently, the incubation and centrifuging is performed at an elevated temperature, preferably a temperature in the interval of about 50°C to about 100°C or, more preferably, between about 60°C and about 90°C.

A well-documented and highly developed particle technology is the so-called nanobead technology. Technologies based on microparticles, silica beads, polystyrene beads, latex beads, glass milk, paramagnetic particles and combinations thereof belong among others to this category of technologies. To observe individual particles and to separate them from each other is however very troublesome and time consuming. See e.g. the FACS apparatus and method described in WO 97/35201. Also specific instruments like microscopes or types of signal amplification means may have to be used. The observation of groups of particles in which each group consists of hundreds or thousands of similar particles, is however done much more easily.

There are at least two distinct properties of such particles that may be used for identification. One is the particle size or diameter. Another is the particle density. Further, the sedimentation speed and other behavioural properties can be used. Particles could also be dyed with colour markers, fluorescent dyes or associated with other markers, such as magnetic or radioactive markers. All these properties may be used to separate a few categories from each other.

However, according to a preferred embodiment of the present invention, density is chosen as the property for characterising the manifold of categories necessary for the microarray technology. The present invention thus presents a microarray based on the density gradient of suspended microparticles upon which reactants are immobilised and able to react with other reactants in solution.

Preferably the density gradient is adapted to the sample to be investigated. According to a preferred embodiment, an adapted gradient is used together with a medium with a function in addition to mere suspension of the particles, such as a medium partaking in the preparation of the sample, e.g. a lysis medium. This way, a pre-treatment step can be easily incorporated in the analysis.

Groups of particles with specific densities are separated from other groups of particles having other densities. On the particle surfaces of each density group of particles, reactant molecules specific for this density group are immobilised. All particle groups are then pooled together in the same vessel containing the solution and the reactant molecules being the counterparts to the immobilised reactants. After a period of time, sufficient for reaction, has passed the different density groups are separated from each other by centrifugation. This results in the particles having the highest density collecting in the bottom of the vessel. The next particle category is the one with the second highest density and so on.

The particles can also be concentrated by gravity settling, diafiltration or evaporation. An important property of the resulting gradient is its discrete distribution with regards to the chosen particle property. The individual groups, divided in respect of particle property, are clearly and distinctly discernible for the chosen method of detection.

According to one embodiment of the invention where the different particle groups are separated by centrifugation, an important step is the fixation or stabilisation of the particle layers achieved by the centrifugation. This can be done, for example, by suspending the particulate carrier matrix in an aqueous medium. After mixing the sample and waiting for the reactions to take place, the sample-matrix-suspension is treated in a manner leading to a clear separation of the particle groups. In an embodiment, where the particle groups have distinct and different densities, centrifugation is the preferred way of achieving this.

One problem, associated with centrifugation of finely divided matter such as cell fragments or, in the present application, groups of particles exhibiting only small density differences, is the vibrations occurring during retardation of the rotor. When the centrifugation has been performed and the rotor speed is slowly lowered, the rotor often vibrates when the speed passes certain critical levels. This is associated with the inherent resonance frequencies of the rotor. These vibrations may upset or at least disturb the fine layers achieved by the centrifugation. In order to overcome this problem, the present inventor discloses the use of a medium and method, wherein the layer structure is fixated upon completion of the centrifugation but before the rotor speed is lowered. This is achieved by suspending the particles in a medium, which can be made to solidify. Examples include aqueous media, which can be frozen, media where a polymerisation process or cross-linking can be initiated with light or radiation, e.g. photo polymerisation.

Preferably the solidification of the medium can be initiated in a non-invasive manner, meaning that the solidification is achieved without harmful influence on the analytes

and/or reactants and without distorting the result of the analysis. In this aspect, freezing of an aqueous medium is suitable for most applications. Photo polymerisation of a polymeric material, containing a suitable initiator, is also suitable.

Examples of particles, suitable for use according to the present invention, include latex beads, polystyrene beads, mineral particles, silica particles and polymers and macromolecules of different origin. A presently available type of particles is for example the Polystyrene Latex Beads obtainable from Sigma Inc. Silicon dioxide particles in the range 0.5 – 10 microns are available from Sigma Inc. Further, metacrylate particles with a density of about 1.2 and resorcinol/formaldehyde particles with a density of about 1.3 – 1.4 can be obtained from Dyno Speciality Polymers AS, Norway.

In general, microparticles are available in many different dimensions and densities. Commercially available silica particles span a range of 0.01 to 20 μm. Latex particles are available at least in the following sizes: 0.05-0.10 μm, 0.10-0.15 μm, 0.15-0.2 μm, 0.20-0.25 μm, 0.25-0.30 μm, 0.3-0.4 μm, 0.4-0.5 μm, 0.5-0.6 μm, 0.6-0.7 μm, 0.7-0.8 μm, 0.8-0.9 μm, 0.9-1.0 μm, 2-2.5 μm, 3-5 μm, and 5-10 μm (Standard Dow Latex).

A conventional fluorimetric or luminimetric reporter system of the type used in current microarray technology may then be utilised to identify the different particle density groups corresponding to different chemical reactions.

Further, by combining the use of microbeads / microparticles of different densities with signal molecules, for example signal molecules also known as fluorophores, higher resolution can be obtained. The number of presently available fluorophores is in the order of 5 to 10. Even when only five fluorophores are combined with a vertical array comprising microparticles of three distinct densities or particle sizes, 15 discernible tests are obtained. The combination of distinct a distinct physical quality of the microparticles, such as density or diameter, and a signalling system, such as fluorophores, allows the manufacture of kits for multiplex analysis having a high resolution. Among other things, the present invention will make possible a more efficient use of the available fluorophores.

The upper limit for the resolution available with vertical microarrays or particulate microarrays according to the present invention is naturally dependent on the number of obtainable distinct fractions and the number of available markers. In addition to fluorophores, also other markers can be used, for example radioactive markers. A conceivable

resolution limit for the system according to the present invention could be in the order of 1000 positions per sample or higher.

Examples

Production example 1:

A suspension of silica particles (a "raw" gradient) having different densities is centrifuged in a medium leading to separation of the particles with respect to different densities. A more or less continuous density gradient is then formed. The centrifugation medium being in liquid phase during centrifugation is solidified, for instance by lowering the temperature or as a result of some chemical reaction, for instance a polymerisation, preferably photoinitiated polymerisation. The solidification should be a reversible process. The centrifugation vessel has preferably an oblong form and may be a plastic tubing closed in its distal end. When the centrifugation medium is solid, the centrifugation vessel is cut into individual segments which may be denoted segment 1, segment 2 etc., where segment 1 is closest to the distal end of the centrifugation vessel segment 2 is second closest to the distal end and so forth. After this, segment 1 and segment 3 and segment 5 etc. are put into one single centrifugation tube. The centrifugation medium within the segments is then re-liquidified and another cycle of centrifugation, solidification, segmentation and assortment of segments is performed. By repeating the process a number of cycles, a discrete density gradient of particles is produced. Density groups are denoted density group 1, density group 2 etc., which denotation are corresponding with segment denotations. For a schematic illustration, see Fig. 2.

On the particle surface of the density group 1 are immobilised reactant molecules specific for this density group. However, if a second set of reactant molecules is immobilised on particles belonging to an adjacent density group, that is density group 2, it is difficult to separate these groups from each other. If instead the second set of reactant molecules is immobilised to particles belonging to density group 3, there will be a space between the density groups making it easier to distinguish between them. An even higher resolution is achieved if density groups 4, 5 or a higher number is used for immobilisation of the second set of reactant molecules. If even further resolution is required, a specific signal molecule may be immobilised on the density group or groups used as spacers between groups carrying reactant molecules.

In this way, a third and further sets of reactant molecules may be immobilised on particles belonging to successively lower density groups separated from each other by spacing regions corresponding to the resolution required for making analysis feasible. A preferred way of analysis is a laser scanning similar to conventional bar code reading systems. The identification of each density group determined from its location relative to adjacent density groups, which may have signals denoting space or may have a lack of signal, also denoting space.

Production example 2:

A homogenous stock of particulate material in solution is divided in multiple aliquots. Preferable, a polymeric material is used. To the first aliquot is added particle size reducing agents, for example acids or bases digesting the particulate material. To the next aliquot, the same agents are added but in a lower dose, alternatively the same dose is added but allowed a shorter time of action. To the next aliquot is added a still lower dose and so one, until one aliquot is reached, to which no size reducing agents are added. Thereafter, size-increasing agents, such as polymers, substrates, coating agents etc are added to the following aliquots in increasing doses. For a schematic illustration, see Fig. 3.

Alternatively, the different aliquots are subjected to heat treatments, ultra sound or chemical / enzymatic treatments influencing the particle size.

After obtaining distinct densities in the different aliquots, these are reacted with suitable reagents and signal molecules. A vertical microarray system can then be assembled by combining particles with desired properties.

Production example 3:

A suspension of particulate matter, comprising a large density variation, is centrifuged and the different layers recovered in separate vessels. In order to achieve sufficient resolution, every second fraction is recovered for use in the same kit or for the same purpose. The intermediate fractions are used for the manufacture of a different kit or for a different purpose. For a schematic illustration, see Fig. 4.

Example 4:

An analysis according to the present invention is performed as follows: a minute amount of sample, e.g. a small volume of a body fluid to be subjected to a diagnostic analysis, is taken from a patient. Optionally, said sample is pre-treated, e.g.

filtered to remove solid particles. A suitable test kit is selected, where the necessary reagents are immobilised to distinct groups of particles. Optionally, the test kit may have to be put in a state of readiness, e.g. by adding a medium or thawing a frozen medium and/or suspending the particles, for example by vortexing the vessel containing the particle suspension. The sample is then added to the reaction vessel, the contents mixed and, after a sufficient time for allowing the reactions to take place, the reaction vessel is centrifuged. When the particle groups have separated in distinct layers, the solidification of the medium is initiated without interrupting the centrifugation. According to one preferred embodiment, an aqueous medium is used and the solidification is achieved by lowering the temperature to or below the freezing or solidification temperature of the suspension. When the suspension has solidified, the centrifugation is interrupted by slowly lowering the rotation speed. Finally, the reaction results are read, e.g. by scanning the layers with a suitable instrument, e.g. a suitably modified bar-code-reader or similar instrument.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventor, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

Claims

1. A microarray system comprising reactants, a carrier matrix and a medium, **characterized** in that said carrier matrix comprises particulate matter in a number of at least three groups, each group having a distinct physical property; said reactants being immobilised to said particles with each one reactant corresponding to one specific group.

2. Microarray system according to claim 1, **characterized** in that the distinct property is chosen among the following: particle size, particle density, sedimentation properties, magnetic properties, radioactive properties, colour, chemical affinity or a combination thereof.

3. Microarray system according to claim 1, **characterized** in that the distinct property is particle density.

4. Microarray system according to any one of claim 1 to 3, **characterized** in that said medium is a medium which can be solidified.

5. Microarray system according to any one of claims 1 to 4, **characterized** in that distinct groups with immobilised reactants are separated by intermediate groups without immobilised reactants.

6. Microarray system according to any one of claims 1 to 4, **characterized** in that distinct groups with immobilised reactants are separated by intermediate groups with specific signal molecules, for example calorimetric fluorophores or luminophores.

7. A method for performing multiplex analysis with a number of reagents and a particulate carrier matrix, **characterized** in that it comprises the following steps:

- adding of a sample to a suspension of carrier particles comprising distinct groups in respect of their physical characteristics, each reagent immobilised to a distinct group,

- bringing the sample in fluid contact with the particles for a time sufficient for the reactions between sample and reagents to take place,

- centrifuging the suspension until the distinct groups separate, and

- determining the reaction result for each reagent or particle group.

8. A method for performing multiplex analysis with a number of reagents and a particulate carrier matrix, **characterized** in that it comprises the following steps:

- adding of a sample to a suspension of carrier particles comprising distinct groups in respect of their physical characteristics, each reagent immobilised to a distinct group,

- bringing the sample in fluid contact with the particles for a time sufficient for the reactions between sample and reagents to take place,

- centrifuging the suspension until the distinct groups separate,

- solidifying the suspension, and

- determining the reaction result for each reagent or particle group.

9. A kit for the performing of multiplex analysis, **characterized** in that said kit comprises a number of particle groups with distinct properties, each particle group carrying a reagent, bound to the particles in that group, a reaction medium for suspending the particles, said reaction medium chosen so that it can be made to solidify after the centrifugation following the reaction between analytes and reagents; and instructions for performing the analysis.

10. Kit according to claim 9, **characterized** in that said medium, aiding in fixing the particle groups after the analysis has/have been performed, is a medium which solidifies when the temperature is lowered.

11. Kit according to claim 9, **characterized** in that said medium is a medium, the polymerisation of which can be non-invasively initiated, e.g. through irradiation.

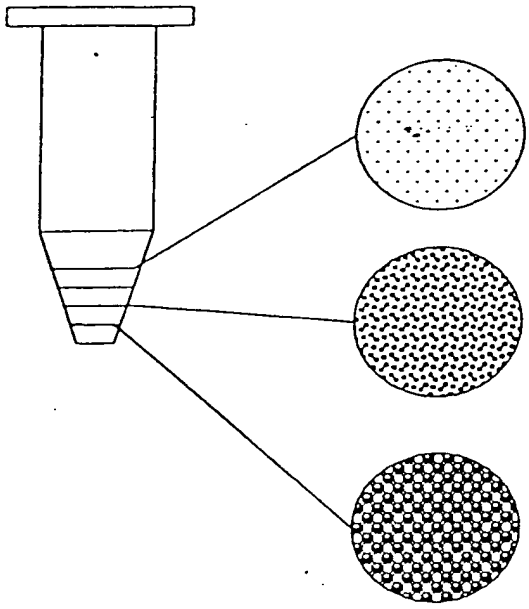

Fig. 1

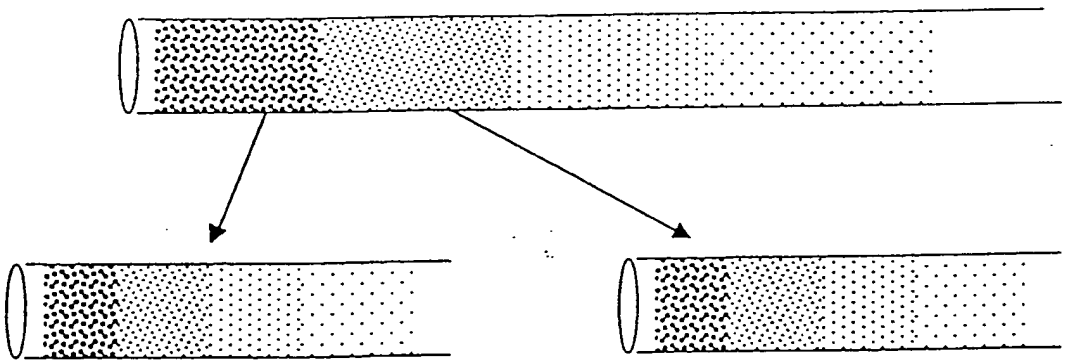

Fig. 2

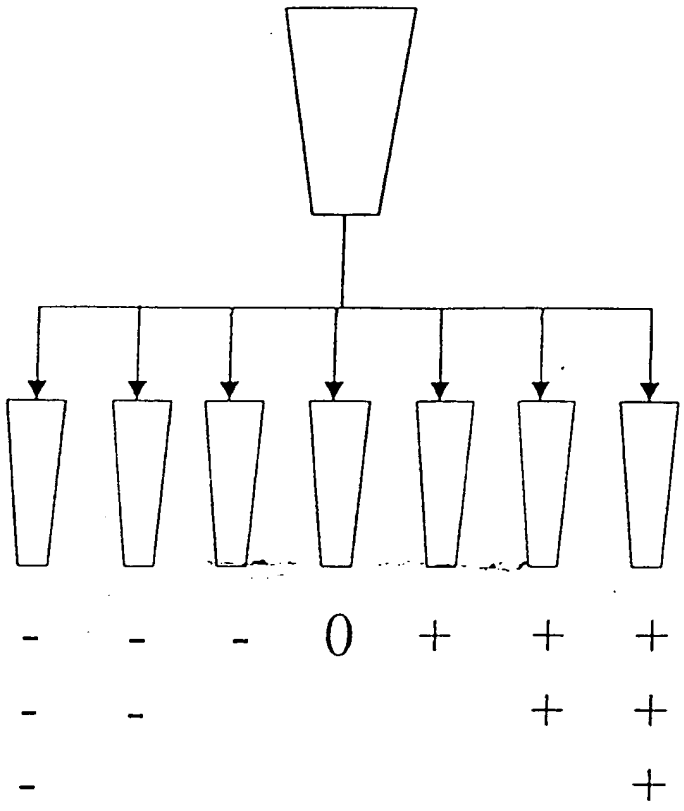

Fig. 3

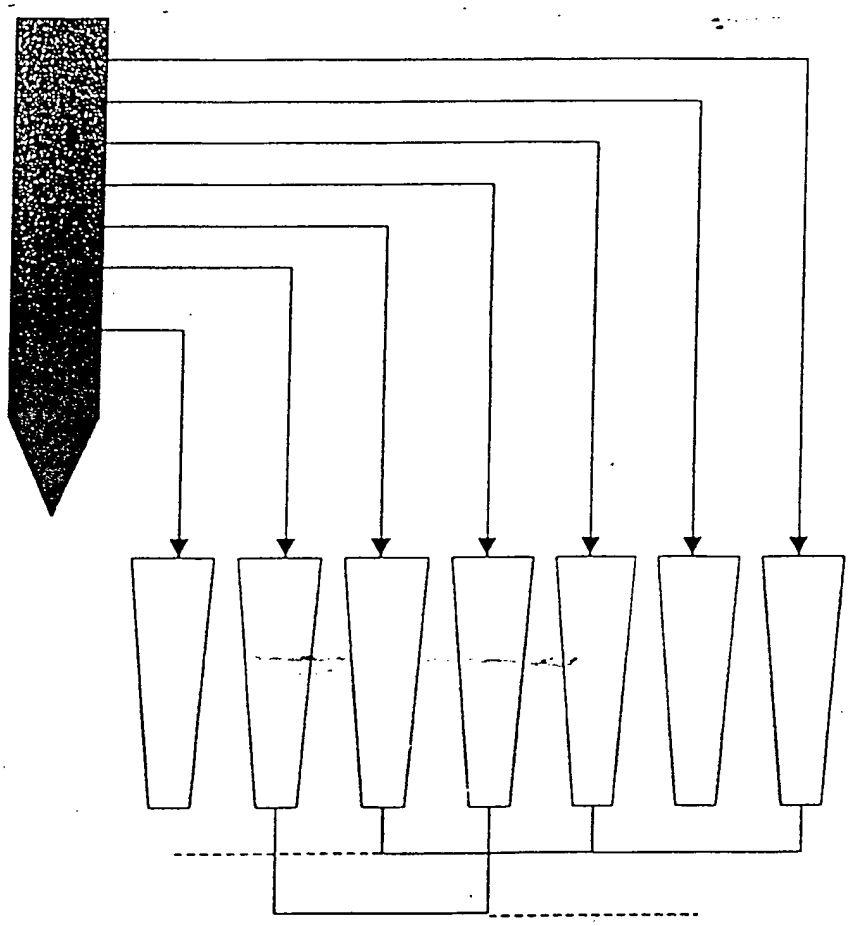

Fig.4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01836

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/543
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9735201 A1 (MARKMAN OFER), 25 Sept 1997 (25.09.97), page 4, line 13 - page 5, line 12	1-2,4-6,9
Y		3,7
	--	
Y	US 4721681 A (BRIAN B. LENTRICHIA ET AL), 26 January 1988 (26.01.88), column 2, line 26 - line 28, claims 1-2, abstract	3,7
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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"E" erlier document but published on or after the international filing date
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2000	14 -02- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustavsson/MN Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/SE 99/01836

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9735201 A1	25/09/97	AU 1938197 A	10/10/97
		CA 2249272 A	25/09/97
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